with the reduced Mmp13 and Ptgs2 mRNA detected in the KO animals. The contribution of Elf3 to load-induced cartilage damage and the identification of targets and mechanism of action in vivo merits further investigation.

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INHIBITION OF CTX-II RELEASE BY CATHEPSIN K INHIBITION IN VIVO BUT NOT IN VITRO SUGGESTS THAT ANTI-RESORPTIVE THERAPY PROTECTS CARTILAGE

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Purpose: Loss of subchondral bone precedes both reduced joint space in human OA and cartilage loss in animal models. This suggests that resorption of subchondral bone might cause or aggravate cartilage loss. Consistent with this, anti-resorptives given *in vivo* suppress release of CTX-II, a peptide fragment generated from cleavage of type II collagen, in several species and prevent disease progression in animal models and in humans. Thus, loss of subchondral bone may destabilise cartilage and induce its degradation by chondrocytes. However, an alternative explanation for suppression of CTX-II has recently been proposed i.e. that osteoclasts themselves release CTX-II when they resorb calcified cartilage, so that there is less release when resorption is inhibited. We therefore tested whether osteoclasts generate CTX-II from cartilage and if this was done in a MMP- or cathepsin K-dependent manner. We used osteoclast-induced CTX-I release from subchondral bone as a comparator. We also compared the *in vitro* data with the effect of a cathepsin K inhibitor *in vivo* on urine CTX-I and CTX-II levels in female beagle dogs subjected to partial medial meniscectomy, an experimental model of OA[1].
Methods: For the *in vitro* study, human osteoclasts were incubated on slices of subchondral bone and overlying calcified and hyaline cartilage ('bone-cartilage') taken from patients with OA. CTX-I and CTX-II levels were measured after a first incubation period for 24 h to establish a baseline release of the two respective biomarkers. The cultures were then incubated with the selective cathepsin K inhibitor MV061194 (300 nM, n=4), or the broad spectrum MMP-inhibitor GM6001 (10 μM, n=4), or vehicle (n=4) for another 24 h. Results were expressed as CTX-I or CTX-II release compared to baseline. In a previously performed experimental dog OA study, female beagle dogs were subjected to partial medial meniscectomy and treated with the selective cathepsin K inhibitor MIV-711 (30 μmol/kg p.o., n=15) or vehicle (p.o., n=15) for 28 days starting the day before surgery. Urine was collected for assessment of CTX-I and CTX-II levels and creatinine. All data are expressed as mean ± SEM.
Results: Osteoclast incubation on the bone-cartilage slices increased CTX-I levels from <LLQ (0.44 nM) to 155 ± 24 nM. CTX-II levels from the same slices were increased from 34 ± 6 pg/ml to 533 ± 23 pg/ml. As expected, the cathepsin K inhibitor MV061194 strongly suppressed CTX-I release (vehicle controls: 76±13% compared to baseline; MV061194: 26±4%). However, there was no change in CTX-II release in presence of the cathepsin K inhibitor (vehicle controls: 92±5% compared to baseline; MV061194: 88±8%). In contrast, the MMP inhibitor GM6001 reduced CTX-II to 45±5% of baseline release. These data however do not exclude that osteoclasts could release other cleavage fragments from type II collagen in a cathepsin K-dependent manner. In female beagle dogs, after 28-day treatment, the selective cathepsin K inhibitor MIV-711 reduced urinary CTX-I levels to 14±1% compared to baseline and CTX-II levels to 20±2%.
Conclusions: Osteoclasts release CTX-I and CTX-II from bone/cartilage explants *in vitro*. The CTX-I release *in vitro* is cathepsin K-dependent while the CTX-II release is not. By contrast, selective cathepsin K inhibition reduces urinary levels of CTX-I and CTX-II in a similar fashion in dog. In dog, these reductions translated into structural benefit on cartilage[1]. Thus, this provides evidence that the reduction in CTX-II *in vivo* by cathepsin K inhibition is probably not caused by direct suppression of osteoclast-evoked CTX-II release. Rather, direct inhibition of cathepsin K-mediated bone resorption protects cartilage, and thereby indirectly reduces the release of CTX-II.
Reference
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KNEE KINEMATIC DIFFERENCES IN ANTERIOR CRUCIATE LIGAMENT DEFICIENT SUBJECTS PRIOR TO RECONSTRUCTION IS ASSOCIATED WITH KNEE T1ρ CARTILAGE RELAXATION TIME AT LONGITUDINAL FOLLOW-UP

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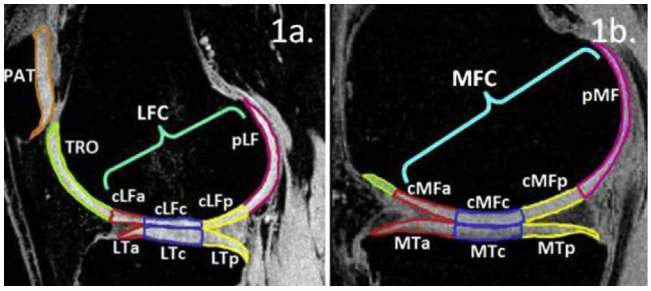
Purpose: This study aimed to identify possible biomechanical markers in gait alterations in anterior cruciate ligament (ACL)-deficient knees prior to reconstruction to predict T1ρ relaxation times.
Methods: Subjects: Motion analysis data were recorded at baseline (n=49, female:male = 20:29, age: 29.63±8.63 years, BMI: 23.8±2.66 kg/m^2) after injury and before ACL reconstruction. Gait Analysis: At baseline, three-dimensional motion analysis was captured while subjects walked at a controlled speed (1.33±0.6667 m/s). Lower-extremity kinematics data were recorded using AMTI force plate (Watertown, MA, USA, sampling frequency = 1000Hz) and Vicon motion capture system (Oxford Metrics, UK, sampling frequency = 250 Hz). Joint external moments and impulses were then computed using Visual3D Software (C-Motion, Germantown, MD, USA). MR Protocol: Of the 49 subjects that conducted motion analysis at baseline, 39 patients returned 12 months following surgery to conduct MR imaging of both knees using a 3 Tesla scanner (GE healthcare) and an 8-channel knee coil (Invivo). Comprehensive cartilage T1ρ relaxation times were calculated using previously validated and published methods by our group. Cartilage was divided into subcompartments to examine regional variations in cartilage health (Figure 1). Mean T1ρ relaxation times were calculated for each global compartment and its corresponding subcompartments.



Statistic Analysis: Pearson product correlation coefficients were calculated between T1ρ relaxation times at the 12-month timepoint and gait characteristics obtained at baseline prior to surgery Variables historically in literature that could be associated with knee osteoarthritis were examined, such as peak knee extension adduction moments and impulses, and hip flexion moments.
Results: In injury knees, baseline lower peak hip flexion moment, lower peak knee extension moment in the 1st half of stance phase and lower peak knee adduction moment in the 2nd half of stance phase were significantly correlated with increased cartilage T1p at 12-months. (Table 1)

Table 1: Baseline motion analysis correlated to T1p at 12-months after ACL reconstruction in injured knees.

Injured Knee at Baseline[c]	T1p[a]
Peak Hip Moment Flexion	cLT: 0.0446 (0.323)[b] TRO: 0.0314 (0.345) cLF.p: 0.0257 (0.334)
Peak Knee Moment Extension (1st half of Stance Phase)	LFC: 0.0383 (-0.333) PAT: 0.00066 (-0.448) TRO: 0.0309 (-0.346) cMF.p: 0.0463 (-0.321)
Peak Knee Moment Addduction (2nd Half of Stance Phase)	cLF.p: 0.0376 (-0.331)

[a] Injured knee 1 year after reconstruction
[b] P-value (R)
[c] External moments

In contralateral knees, baseline lower peak hip flexion moment Lower peak knee extension moment in the 1st half of stance phase and lower peak knee adduction moment in the 2nd half of stance phase were significantly correlated with increased cartilage T1p at 12-months. (Table 2)

Contralateral Knee at Baseline[c]	T1p[a]
Peak Hip Flexion Moment	TRO: 0.0482 (0.316)
Peak Knee Moment Extension (1st half of Stance Phase)	LFC: 0.0499 (-0.317) cMF.c: 0.0203 (-0.370) TRO: 0.278 (-0.352)
Peak Knee Moment Addduction (2nd Half of Stance Phase)	pMF: 0.0274 (-0.306)

[a] Injured knee 1 year after reconstruction
[b] P-value (R)
[c] External moments

Conclusions: The correlations observed between joint loading at baseline and knee cartilage T1p at 12-month after surgery indicate that baseline joint loading in the ACL-deficient state may be a significant indicator and predictor of cartilage T1p 1 year after surgery. Our results showed that there exist similar correlations in both the injured and contralateral knee to cartilage health 1 year after surgery. A possible explanation for this result is that certain individuals who characteristically walk with greater loading in the knee during gait, specifically with a higher extension and adduction moment are at higher risk of causing degenerative changes in their cartilage, while higher loading in the knee could potentially lower the risk of degenerating cartilage. Cartilage T1p has been shown to show degenerative changes in cartilage health as soon as 1 year following ACL reconstruction, and these results indicate that measuring hip and knee loading prior to ACL reconstruction may predict cartilage health after ACL reconstruction. This study was funded by NIH/NIAMS P50 AR060752.

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ENDOGENOUS HYDROGEN SULFIDE PRODUCTION IS REDUCED IN OA CARTILAGE. POSSIBLE CONTRIBUTION TO THE PATHOGENESIS OF OA

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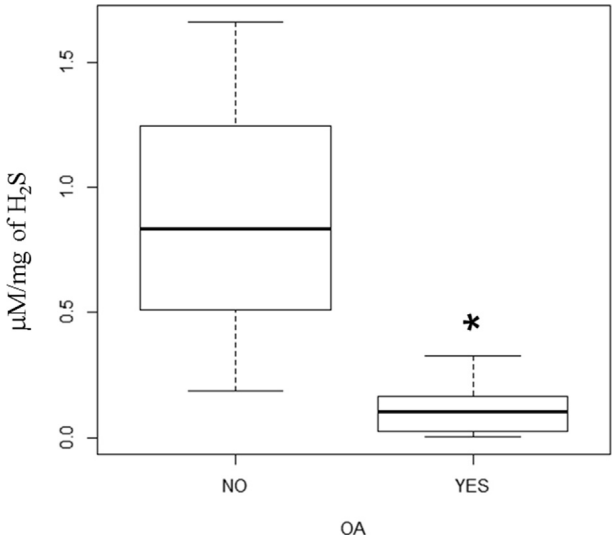
Purpose: Hydrogen sulfide (H2S) is a newly discovered endogenous gas (gasotransmitter) that is involved in many physiological processes. Recent studies have also shown that some in pathologic situations such as hypertension, cardiovascular disease or diabetes type II, H2S endogenous levels are significantly reduced. We hypothesize that this might be the case also in osteoarthritis (OA). Therefore the purpose of this study was to analyze the levels of hydrogen sulfide in blood serum, its production from cartilage and the expression of its synthesizing enzymes in the joint, comparing OA and non OA samples.
Methods: Tissue (cartilage, synovial membrane and subchondral bone) and blood samples were obtained from patients of the Orthopedic or Rheumatology Services of the University Hospital A Coruña (CHUAC), after written informed consent. The mRNA expression of the H2S production enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CTH) and mercaptopyruvate sulfurtransferase (MPST) was analyzed in cartilage, synovial membrane and subchondral bone. Based on the obtained results, the presence of the enzymes was visualized in cartilage through immunohistochemistry and quantified with appropriate software (ImageJ). Hydrogen sulfide concentration was measured using a selective ion microelectrode. Direct H2S concentration in 200 μL of fresh blood serum was measured after incubation with 200 μL of an antioxidant buffer (AB) for 1h. Likewise, H2S production from cartilage was measured by incubating cartilage discs (6mm in diameter, 3mm height) in 200 μL of the same AB for 2 h. In both cases results in mV were translated into H2S concentration (uM) using a calibration curve, and normalized per ul of blood serum or mg of cartilage.
Results: With respect to the H2S production enzymes, we found that CBS and CTH were expressed in all tested joint tissues. We found a tendency for CBS to be reduced in the OA cartilage, but no statistically significant differences were detected in these enzymes in all tissues. However, interestingly, MPST was only found to be expressed in cartilage, and its mRNA expression was significantly lower in OA cartilage with respect to non OA cartilage (Image 1). Immunohistochemistry also showed decreased levels of this enzyme in cartilage. On the other hand, statistically significant differences were also found in the H2S production measured from OA with respect to non OA cartilage samples. Indeed, OA cartilage H2S production was 0.13 ± 0.03 uM/mg, n=11 while that of healthy cartilage was 0.92 ± 0.42 uM/mg, n=3 (mean ± SEM, $p<0.05$). Conversely, these results were not reproduced in blood serum samples, where no statistically significant differences were found (OA serum: 0.37±0.05 uM/μL, n=27 vs. N serum: 0.31±0.04 uM/μL, n=31; mean ± SEM).
Conclusions: The current results show, for the first time, that the levels of the endogenous gasotransmitter H2S are reduced in OA cartilage with respect to normal controls. Since endogenous H2S exerts anti-inflammatory and anti-oxidant effects, the lower concentration and production found here, might be contributing factors to the pathogenesis of OA.



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SPONTANEOUS DEVELOPMENT OF SCLEROTIC CHANGES OF SUBCHONDRAL BONE AND CARTILAGE DEGRADATION IN ENDOTHELIN-1 OVEREXPRESSION TRANSGENIC MICE UPON HIGH FAT/HIGH CHOLESTEROL DIET

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Purpose: Osteoarthritis has been found to be well correlated to metabolic syndromes like hypertension and obesity but being a multifaceted degenerative disease, many of the actual biochemical pathways remain unsolved.
We have previously demonstrated the effect of endothelin-1, a potent vasoconstrictor that contributes to hypertension, in the development of osteoarthritic changes in the subchondral bone. This time, we would like to investigate the interplay and biochemical roles of obesity and endothelin-1 in osteoarthritis and propose the use of obese endothelin-1 overexpressing mice as an OA model.
Methods: Male, 35-week-old endothelin-1 overexpressing (TET) mice and their non-transgenic littermates were used in this study. Half of each group was fed with high fat, high cholesterol chow to induce obesity, and the other half was fed with normal chow.
Knee joints were harvested and fixed in 10% formalin solution immediately after sacrifice. Micro-CT scans of the knee joints were performed before tissue processing. Subchondral bone structures were analysed in CTAn Software.
Samples were embedded for paraffin sectioning at 5μm at sagittal plane.
Safranin-O staining was done to evaluate the cartilage degeneration indicated by loss of glycosaminoglycan content.
Immunohistochemical staining was performed using rabbit primary antibodies coupled with Streptavidin-HRP developed with DAB substrate, and counterstained with Harris Haematoxylin.
Results: TET mice fed with high fat & high cholesterol diet were found to have higher bone volume fraction (43.3% vs 36.3%) and lower bone